Supporting Information

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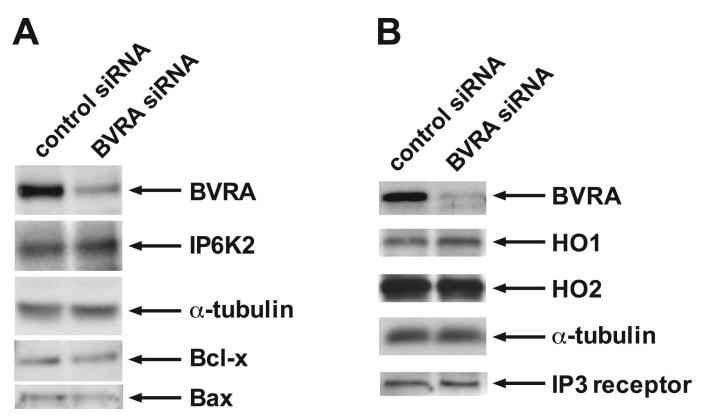


Fig. S1. Selective depletion of BVR with siRNA. HEK 293 cells were transfected with BVR 180 siRNA and BVR180c control siRNA, then immunoblotted with the specified antibodies at 72 h. (A) Similar levels of apoptosis-related proteins Bax, Bcl-x, and IP6K2 following BVR depletion. (B) Similar levels of HO1 and HO2 following BVR depletion.

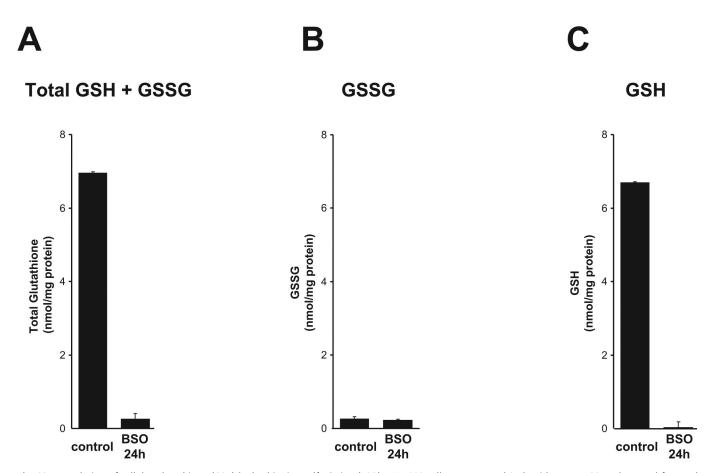


Fig. S2. Depletion of cellular glutathione (GSH) by buthionine sulfoximine (BSO). HEK 293 cells were treated 24 h with 1 mM BSO and assayed for total glutathione levels (A), oxidized glutathione, GSSG (B), and reduced glutathione (C). Glutathione levels rapidly decrease by 96% at 24 h and 99.9% at 48 h.

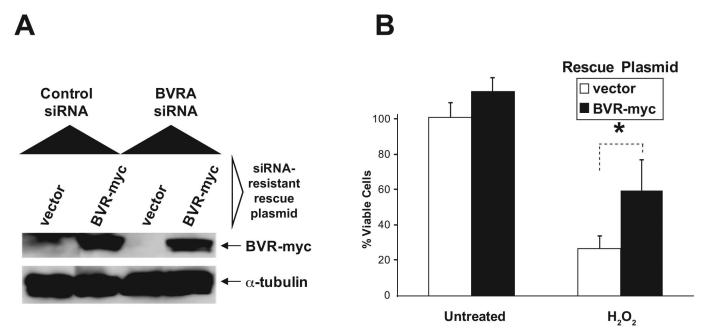


Fig. S3. Rescue of siRNA-depleted BVR with exogenously transfected BVR. (A) Rescue of siRNA-depleted BVR by exogenous plasmid. At 24 h after transfection of siRNA, cells were retransfected with pcDNA3 (vector) or a pcDNA3-BVR-myc harboring silent mutations rendering it resistant to siRNA oligo 180. Exogenously transfected BVR was visualized with myc-epitope-specific antibody at 72 h. (*B*) Exogenously transfected BVR rescues death from BVR depleted cells. At 72 h cells were treated with 200 μ M hydrogen peroxide (H₂O₂) and viability assessed by MTT. Data represent means \pm SEM of triplicate determinations. *, P < 0.05 by t test.

A B

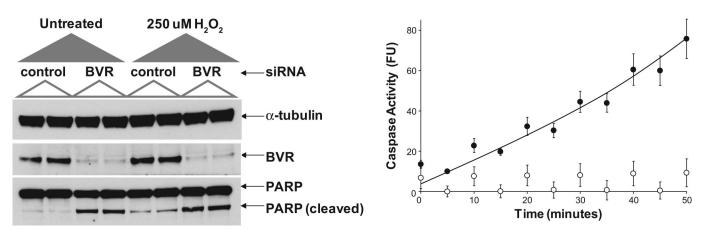


Fig. S4. Cell death following BVR depletion is apoptotic. (A) siRNA depletion BVR increases poly(ADP-ribose) polymerase (PARP) cleavage, a marker of apoptosis. HEK293 cells were transfected with BVR or control siRNA oligonucleotides and cell lysates immunoblotted for BVR and PARP. (B) siRNA depletion of biliverdin reductase (BVR) increases caspase activity, a marker of apoptosis. Caspase 3/7 activity was measured with fluorescent substrate Z-DEVD-R110. Means of quadruplicate samples shown are representative of 2 independent experiments. *, P < 0.05 vs. control for all points except time zero.